The fungus *Leucopaxillus cerealis* newly recorded from Australia

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Abstract
Since June 2007, *Leucopaxillus cerealis* (Fungi: Agaricales) has been observed growing under eucalypts in garden beds at the Monash University Clayton campus, in suburban Melbourne, Victoria. A description of the fungus is given, which represents the first record of the species from Australia. Significant variation in the appearance of the fruit-bodies was observed between occurrences in different years, possibly because of different weather conditions. The antibiotic properties of this species could have important implications for Australian environments. (*The Victorian Naturalist* **129** (5) 2012, 160–166)

Keywords: Leucopaxillaceae, *Leucopaxillus albissimus*, Eucalypts, Tricholomataceae, *Phytophthora cinnamomi*

Introduction

Leucopaxillus is a genus of agarics (gilled fungi) characterised by the fleshy fruit-bodies lacking a partial or universal veil; often with decurrent lamellae and with a white spore print. Microscopically, spores are amyloid in Melzer's reagent, and smooth or with a warty ornamentation. Molecular studies have shown that *Leucopaxillus* belongs to the tricholomatoid clade of the Agaricales (Matheny *et al.* 2006), and is considered to be saprotrophic, not mycorrhizal (Tedersoo *et al.* 2010).

Three species of *Leucopaxillus* have been reported from Australia (May *et al.* 2006): *L. amarus* (Alb. & Schwein) Kühner, *L. eucalyptorum* (Cleland) Grgur. and *L. lilacinus* Bougher. These three species all have a brown, grey or purple pileus (Bougher and Syme 1998; Fuhrer 2005). Incidentally, *Leucopaxillus amarus* as used by most authors is more correctly called *L. gentianus* (Quél.) Kotl. (Knudsen & Vesterholt 2008).

A fungus with a white pileus matching *Leucopaxillus cerealis* (Lasch) Singer was first observed in June 2007 growing under eucalypts and other plants in a garden bed at the Monash University Clayton campus, in suburban Melbourne, Victoria. This is the first record of this species from Australia. Members of the *L. cerealis* complex are usually found in the Northern Hemisphere where they are widespread but uncommon, generally growing under conifers or broad-leaved trees, including exotic eucalypts (Arora 1986, as *L. albissimus* (Peck) Singer). We report below on the characters and identification of the Australian material and discuss the significant variation in appearance that we observed over different years.

Materials and methods

Macroscopic descriptions were made from fresh specimens. Microscopic characters were examined by standard techniques, using a Motic BA200 optical microscope with a trinocular head, and a Canon D1000 digital camera. The microscope was calibrated by using an Olympus objective micrometer with 10 µm divisions. Mounts for microscopical examination were made from dried and fresh material; measurements were made in 5% potassium hydroxide solution or in Congo Red with 10% ammonia. Melzer's reagent was used to determine the amyloid reaction of the basidiospores. All measurements were carried out on digital images using a public domain software package called ImageJ. The drawings of spores, basidia, and cystidia were made by tracing over digital micrographs in Photoshop™. In 2008, 2010 and 2011, specimens were collected and some of the material was deposited in the National Herbarium, Royal Botanic Gardens, Melbourne. No fruit-bodies emerged in 2009, because of the dry weather.

Taxonomic description

Pileus 50–100 mm or more broad; when young convex with an inrolled margin, expanding to nearly plane, with age it can become centrally depressed (Fig. 1), margin frequently ribbed on young fruit-bodies (Fig. 2); surface dry, dull, glabrous when young and moist, becoming scaly, tomentose or fibrillose and cracking when dry and with age; colour when very young

Fig. 1. Fruit-bodies can be over 100 mm across with uplifted margin, and centrally depressed.

Fig. 2. Young white caespitose fruit-bodies, with ribbed margins.

pure white, with age becoming tinted yellowish brown, darker in the centre, and occasionally turning light tan (Fig. 3); context white, becoming cream-coloured with age, does not bruise.

Lamellae attachment subdecurrent to decurrent; often with decurrent lines or ridges extending down the stipe (Fig. 4); close or crowded, thin, sometimes forked, sometimes anastomosing; can be separated easily from the stipe (Fig. 5) and also from the context of the

Fig. 3. Pileus becoming light brown to tan with age.

pileus; colour white at first, becoming buff with age or on drying out. Lamellulae present.

Stipe central; generally up to 60 mm or more long and 30 mm thick; stout, cylindrical but often enlarged at the base or in the middle; surface smooth to finely scaled or fibrillose; colour white when young, becoming pale buff with age; growing from copious white mycelium that combines with the mulch and soil to form a thick, firm mat.

Flesh up to 17 mm or more thick in large specimens, white, firm.

Odour when fresh, none to very mild but unpleasant; dried material has a very strong unpleasant odour.

Taste VH found the taste to be very bitter and unpleasant, while JH thought that it was only mildly bitter.

Basidiospores 6.3–7.5 × 4.7–5.3 µm (excluding ornamentation), ellipsoid to almost oval, hyaline, often with one oil drop, with ornamentation of scattered, strongly amyloid warts (Fig. 6).

Basidia 35–43 × 8–9 µm, narrowly clavate, four-spored, often with a clamp connection at the base (Fig. 6).

Cystidia cheilocystidia and pleurocystidia present, filamentous, often with short branches, often septate (Fig. 6); not easy to find in some specimens, but abundant in others.

Pileipellis consisting of appressed interwoven hyphae with clamp connections. Clamp connections were found in all tissue.

Fig. 4. Clitocyboid fruit-body.

Fig. 5. Swelling of stipe due to rain after a short dry period.

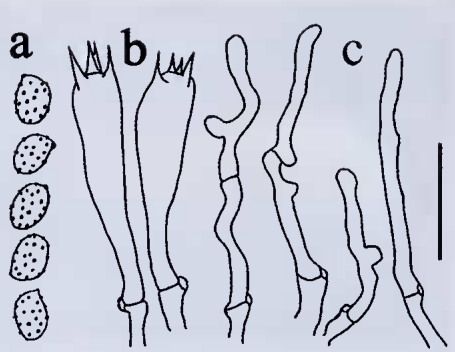

Fig. 6. (a) Spores (b) Basidia (c) Cheilocystidia. Scale bar = 20 µm.

Fig. 7. Naucoroid/clitocyboid fruit-body.

Growth habit solitary, scattered or in caespitose clusters in a 'fairy ring' in thick (up to 100 mm deep) leaf litter or mulch under eucalypts and other plants; stature naucoroid/clitocyboid (Fig. 7) or clitocyboid (Fig. 4).

Growing season generally April to June if weather conditions are suitable; however, in 2011, for the first time, a few new fruit-bodies emerged as late as 26 August.

Habitat and phenology of *L. cerealis*

The original location at Monash University was near the south-east corner of the intersection of Normanby Road and East Ring Road. The vegetation in this garden bed consists principally of *Eucalyptus* spp., *Grevillea* spp. and Gymea Lily *Doryanthes excelsa*. Most of the eucalypts in this area are Spotted Gum *E. maculata*, and it is under this species that the *L. cerealis* grows. No conifers are present.

A large 'fairy ring' of *L. cerealis* was first observed in deep leaf litter under one *E. maculata* in June 2007. There had been significant rainfall and the fruit-bodies had grown to a diameter of more than 100 mm. No collection was made because all fruit-bodies were old when discovered.

In 2008 there was initial good rain, allowing the formation of a number of naucoroid/clitocyboid fruit-bodies (Fig. 7), but then the season stayed dry, causing the fruit-bodies to become stunted and deformed. In the same year a single small fruit-body emerged under a second *E. maculata*, but dried out before it had a chance to mature.

In 2009 conditions were much drier, and although some mycelium developed amongst the leaf litter under the first *E. maculata*, no fruit-bodies were observed.

In 2010 there was some rain but the fruit-bodies stayed small and developed predominantly in several caespitose clumps, forming a 'fairy ring' (Fig. 8) under the first *E. maculata*. Fruit-bodies also emerged under a third *E. maculata*. In the same year, a 'fairy ring' of *L. cerealis* fruit-bodies was observed in mulch in a second garden bed, on the south side of the multi-level car park, about 730 m south-west of the first site. The vegetation at this new site consists of various *Eucalyptus* spp., *Kunzea baxteri* and a Cypress-pine *Callitris* sp.

In 2011 rain fell much more frequently, and several fruit-bodies, some more than 100 mm in diameter, developed under the eucalypts at the first site. However, dry periods between rain events again caused most fruit-bodies to become stunted and contorted, with cracked yellowing pilei (Fig. 9) and split or swollen stipes (Fig. 5). Some *L. cerealis* fruit-bodies, along with *L. eucalyptorum*, were observed at the second site. In the same year, fruit-bodies of *L. cerealis* were found at two new sites on the north side of the Campus Centre, about 360 m south-east of the second site, where no conifers were present. One group of fruit-bodies was

Fig. 8. 'Fairy ring' of caespitose clumps of fruit-bodies.

Fig. 9. Pileus becoming severely cracked in dry weather.

growing under a Red Flowering Gum *Corymbia ficifolia* and the other about 30 m away under a Warted Yate *E. megacornuta*.

In all cases, the *L. cerealis* fruit-bodies were slow growing and lasted for at least two months. When young they were white, but with age they became yellowish, then finally rusty brown; their smell became disagreeable, and the surface of the pilei became cracked as they dried. From these observations it is evident that the fruit-body size, coloration, growth habit and smell are highly dependent on environmental factors.

Discussion

The micro-morphological characteristics of the Australian collection (particularly the amyloid nature of the warts on the spores, and the filamentous cheilocystidia), as well as the macro-morphological features, match the description of *Leucopaxillus cerealis* by Singer and Smith (1943: as *L. albissimus*) and Knudsen and Vesterholt (2008). Singer and Smith (1943) had treated *L. albissimus* as a very variable species, including several forms and varieties. Singer (1986) updated the correct name of *L. albissimus* to *L. cerealis*, and listed numerous synonyms. Knudsen and Vesterholt (2008) regarded *L. cerealis* as a species complex, including *L. albissimus*, *L. paradoxus* (Costantin & L.M.Dufour) Boursier and *L. piceinus* (Peck) Pomerl. In addition, Knudsen and Vesterholt (2008) treated *L. cutefractus* Noordel. as a similar but distinct species, separated by the pileus cracking with age. Another species that belongs to the *L. cerealis* complex is *L. monticola* (Singer & Smith) Bon (Vizzini and Contu 2009).

A revision of *Leucopaxillus* was carried out by Singer and Smith (1943) who recognised species based primarily on fruit-body structure, taste, odour and subtle variations in microscopic characters. Since then, no other major taxonomic studies have been carried out on the genus. In particular, there are no studies describing the boundaries of various *Leucopaxillus* species using molecular data.

While the Australian material matches well to the *L. cerealis* complex, difficulty arises when trying to place it into one of the recognised taxa within that complex. Some of these taxa are recognised at varietal level, for example by Singer and Smith (1943), and some as independent species. Microscopic characters are all very similar in members of the *L. cerealis* complex (Table 1). In particular, for most species within this complex, spore and basidia sizes, and the cheilocystidia and pleurocystidia are virtually identical. This leaves the macro-morphological features as the main criterion with which to place the Australian material within particular taxa in the *L. cerealis* complex.

Leucopaxillus cerealis. As described by Singer and Smith (1943: as *L. albissimus* var. *typicus*) *L. cerealis* in the strict sense is characterised by its convex to expanded white pileus with a ribbed but sometimes smooth margin. The pileal surface is smooth or rarely faintly rimose-areolate in age. The odour is aromatic or sweet, and the taste farinaceous-bitter. The lamellae are white, crowded or subcrowded, sometimes forked at the base, mostly anastomosing, adnate with a decurrent tooth or simply decurrent, some with short rib-like proliferations down the apex of the stipe, ribs not anastomosing, moderately broad (to 6 mm), lamellulae abruptly rounded at inner extremity. The stipe is 40–70 mm long, 7–15 mm thick, and the base often bulbous with adherent remnants of leaves, white-myceloid, not strigose, solid, white, subglabrous or somewhat fibrous-scabrous, especially at the apex. Cheilocystidia are rare or lacking, and pleurocystidia are absent.

In many of the Australian specimens, the macro-morphological features match closely with those of *L. cerealis* in the strict sense, although none of the specimens had an aromatic odour. Cheilocystidia and pleurocystidia were

Table 1. Spore and basidia sizes for some *Leucopaxillus* spp. belonging to the *L. cerealis* complex. (1) Singer and Smith 1943, (2) Noordeloos 1995, (3) Singer and Smith 1947, (4) Vizzini and Contu 2009

Species	Spore size µm	Basidia size µm
Australian collection	6.3–7.5 × 4.7–5.3	35–43 × 8–9
L. cerealis (as *L. albissimus* var. *typicus*) (1)	5.5–7.5(–8.5) × 4.2–5.5	24–36 × 6.5–8.5
L. paradoxus (as *L. albissimus* var. *paradoxus*) (1)	5.0–7.5 × 3.3–4.8	21–41 × 4–8
L. piceinus (as *L. albissimus* var. *piceinus* (1)	5.5–8.0 × 4.5–5.0	33–41 × 6–8
L. cutefractus (2)	6.5–8.0 × 4.5–6.0	24–52 × 8–11
L. monticola (3)	6.5–8.0 × 4.5–5.0	20–50 × 4–5
L. monticola (4)	7.0–8.0 × 4.5–5.2	30–40 × 8–10

present in all specimens, but in some samples examined they were very rare. The density of cheilocystidia and pleurocystidia seems to vary significantly, and may be influenced by environmental conditions.

L. paradoxus. This taxon, as described by Singer and Smith (1943: as *L. albissimus* var. *paradoxus* (Costantin & L.M.Dufour) Singer & A.H.Sm.) is recognised mainly by its convex to plane, sometimes centrally depressed pileus with the central area coloured creamy white, or tinted with an ochraceous cream colour. The pileal surface is sometimes faintly rimose-areolate, the space between the areolae being paler. The taste is also significant in that it is mildly bitter or unpleasant. The stipe is white and fibrillose. Cheilocystidia and pleurocystidia are absent.

Morphologically, some of the Australian specimens fitted this description, the main difference being that cheilocystidia and pleurocystidia were present, though very rare in some samples.

L. piceinus. Diagnostic features that differentiate this taxon, as described by Singer and Smith (1943: as *L. albissimus* var. *piceinus* (Peck) Singer & A.H.Sm.), from other members of the complex are the disagreeable, pungent odour, strong bitter or unpleasant taste, surface of the pileus appearing matted-fibrillose under a lens, margin frequently ribbed or grooved, and stipe often with anastomosing ridges at the apex. Cheilocystidia and pleurocystidia are very scattered.

It can be seen from our species description that the Australian specimens match the morphological characters of *L. piceinus*.

L. cutefractus. The important features considered by Noordeloos (1995) to differentiate this species from others in the complex are the numerous cheilocystidia, the cracking of the ochraceous tomentose pileal surface with age, and the normally mild and/or unpleasant taste. Many of the Australian specimens (e.g. the specimen in Fig. 9) have features that match those of *L. cutefractus*.

L. monticola. According to Singer and Smith (1947: as *L. albissimus* var. *monticola* Singer & A.H.Sm.) and Vizzini and Contu (2009) this taxon has a very similar macro-morphology to that of *L. cerealis* var. *piceinus*. However, it is separated by its pleasant aromatic odour and mild taste when fresh, and the disagreeable and distinctive odour when dried, and also its abundant cheilocystidia.

At various times, many of the Australian specimens matched the morphology of this species very well, although none had an aromatic odour when fresh.

Conclusion

Leucopaxillus cerealis has been observed growing in the same general location for four years, during which the climatic conditions have varied significantly. It is clear that the morphology of this species is affected by environmental factors. When there is plenty of rain, the fruit-bodies are usually large, white and smooth. When a period of significant growth is followed by dry weather, the pilei become areolate-cracked. When there is little rain during early growth, the fruit-bodies normally stay small, smooth and whitish. Weather alternating between dry and wet periods causes the fruit-bodies to become quite distorted, and often the stipe swells up. A lot of variability in the abundance of cystidia has also been noted, and the taste seems to depend to some extent on the taster. Because this species is very variable, and the form is so dependent on the weather conditions and age of the fruit-bodies, a chance sighting of any one of its various forms could easily place it in one of the species mentioned above.

The lack of any DNA sequence data means that the best that can be done at present is to call the Monash species *L. cerealis* because most of the observed specimens match the characteristics described for this species. Our observations over several years suggest that some names for members of the *L. cerealis* complex may represent variation caused by environmental factors.

Much research on species belonging to the *L. cerealis* complex has been carried out by molecular chemists attracted to this fungus because of the wide range of compounds that are in its tissue. It has been shown that some of the compounds produced by this fungus have medically useful antibiotic and anticancer properties (Sakai *et al.*1955; Pfister 1988). The fruit-bodies are slow to grow and even slower to decay (Arora 1986), possibly because the antibiotic substances they produce prevent, or slow down, attacks by invertebrates and bacteria. Marx (1969a, 1969b) found that *L. cerealis* var. *piceinus* produces antibiotics antagonistic to *Phytophthora cinnamomi*; this could have important implications for some Australian environments.

Acknowledgements

Our grateful thanks go to referee Dr Tom May, Senior Mycologist, Royal Botanic Gardens Melbourne, for many helpful suggestions which improved the manuscript.

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Received 24 November 2011; accepted 12 July 2012

Eighty-nine Years Ago

POLYPORUS MYLITTAE ... For fifty years or so the substance known as Native or Blackfellows' Bread had been discussed, but no definite conclusion had been come to regarding it until October, 1892, when Dr. M. C. Cooke, the well-known mycologist, announced in the Gardeners' Chronicle that at last the complete plant had been received, and that he intended to name it as *Polyporus mylittae.*

... It seems to me that the fungus may be more common than is usually supposed, as from its mode of growth it might often be gathered, even by a field naturalist, without any suspicion that it was attached by perhaps a rather long stem to the underground substance known as Native Bread. The specimen exhibited to-night grew from a broken sclerotium which was soft when I got it, and was put on a shelf as a curio. I did not happen to notice the growth, which is of velvety appearance and lemon-coloured, for about a fortnight afterwards, so whether it grew while the mass was soft, or after it had hardened, cannot say. As it is so rare, it is my intention to hand the specimen to the National Herbarium, which, I understand, does not possess a specimen.—F. G. A. BARNARD.

From *The Victorian Naturalist* **XXXIX**, pp. 159–160, March 8, 1923